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THE LOCATION AND LONGEVITY IN CALVES OF *BACTERIUM ABORTUM* INGESTED WITH MILK, AND ITS EFFECT ON THE AGGLUTINATION TITRE OF THEIR BLOOD.

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INTRODUCTION AND REVIEW OF THE LITERATURE

The discovery of the elimination of *Bacterium abortum* in the milk of a considerable percentage of cows infected with this organism was made by Schroeder and Cotton[13] in 1911. They found the infection to persist in this location in some cows for a period of years, and this has been confirmed by investigators in various parts of the world. Its presence in the mammary secretion affords an ideal opportunity for abundant ingestion of the organism by the offspring during the first months of its life.

In abortion-infected herds, it is very common to see the disease manifest itself in heifers with their first pregnancy even after premature expulsion of the fetus has practically ceased in the multiparous females owing to the establishment of herd immunity. This naturally suggested that, if the organism could remain viable in the udder of infected cows for a period of five to seven years, or longer, it might remain viable in the bodies of calves during the one or two years from the milk-drinking period until puberty and breeding took place, and be responsible for the abortions in these animals with their first pregnancies. While this hypothesis was reasonable, it was advanced in such a way as to lead many to believe it to be an established fact. Moreover to it was added a second hypothesis, viz., that the only way

* C. M. Carpenter took an active part in this work during the first six months, from December, 1922, to June, 1923, and published a report of this early part of it in The Cornell Veterinarian, 19:16–31.

to prevent infection and subsequent abortion in primiparous animals raised in infected herds was to feed them with pasteurized milk throughout the milk-drinking period of their lives. Were this the true state of affairs regarding the disease, great practical difficulties would stand in the way of controlling this method of infection. A considerable amount of investigational work has been carried out during the last few years to establish the true status of this phase of *Bacterium abortum* infection.

Williams has been the most persistent advocate of this theory, although during recent years he has modified his views. In 1924, in discussing the transmission of permanent genital infection from parent to offspring, he and his co-workers[19] are quoted as follows:

"It is stated[5] that the bacillus abortus infection acquired by the fetus in utero or by the newborn, does not persist until breeding age and cause injury. This claim has led some to believe that no genital infection may be so transmitted. We make no claim that the *B. abortus* infection is thus transmitted—that is not our theme."

Before the full significance of this question became apparent, Mohler and Traum,[9] in 1911, obtained positive complement fixation and agglutination reactions from a prematurely-born calf which died of scours when ten days old in an infected herd. Experimentally, however, they were unable to produce any noticeable ill effects on two three-day-old calves that were each fed with 90 mils of a *Bacterium abortum* bouillon culture in milk for a period of three days.

McFadyean, Sheather and Minett,[8] in 1913 reported an experiment in which a cow was given *Bacterium abortum* intravenously to see whether the calf would become infected by means of the milk or from contact. The blood of the cow became positive, but the calf remained uniformly negative up to the time of separation from the cow and as long thereafter as it was tested. No attempt was made to demonstrate *Bacterium abortum* in the milk.

In 1916, Schroeder and Cotton[14] demonstrated the high agglutinating power of colostrum from cows with infected udders, followed by a rapid decline in titre as milk took the place of colostrum. Blood of new-born calves suckling dams whose udders were known to be heavily infected with *Bacterium abortum*, although having about the same agglutination titre as that of the dams, still showed a rapidly declining agglutination reaction. They found that *Bacterium abortum* ingested with milk does not seem to penetrate deeply or abundantly into a calf's body. They were able to engender agglutinating proper-

([5] Schroeder, E. C. Investigations on Bovine Infectious Abortion, Rep. U. S. Live Stock Sanitary Asso., 1921, p. 65.)

ties in the blood of calves by injecting them with abortion organisms, but such injections had to be repeated from time to time, otherwise the agglutinating properties of their blood serum disappeared.

In the same year, Eichhorn and Potter,[4] in field observations on a large dairy herd, noted the history of forty-one animals born in 1911 but kept separate from adult cattle after they could sustain themselves, although having had ample opportunity to acquire the infection by association with infected mothers and the ingestion of infected milk. Nine aborted in 1915 after producing two normal calves each and one, three calves. One aborted twice, having produced one calf, one aborted three times, and one, once, without living offspring. Three aborted in 1914 after having calved normally. They state that if it is a fact that calves acquire the infection shortly after birth and the organism remains latent in the animal's body awaiting pregnancy, it is strange that abortion should not have occurred before the second, third or even the fourth pregnancy.

In 1917, Huddleson[5] reported the feeding of new-born calves with milk that reacted positively to the agglutination test, and the presence of *Bacterium abortum* was further confirmed by guinea pig inoculation. The calves were separated from their dams shortly after birth and fed the infected milk, from a pail, twice daily. The effect of the feeding was studied by means of the agglutination and complement fixation tests upon the blood sera. Eleven calves were fed naturally-infected milk and six were fed non-infected milk, the latter being allowed to suckle their dams for three days before being separated. Non-infected milk consisted of milk that did not show the presence of *Bacterium abortum* agglutinins. One control animal was fed pasteurized, naturally-infected milk and a second was given non-infected milk plus 5 mils of a 48-hour bouillon culture of *Bacterium abortum* with each feeding.

The effect of the feeding was studied by recording the presence of agglutinating or complement fixing substances in the blood of the animals, and the author concludes that such bodies are very rarely demonstrated in the blood of calves as a result of ingesting naturally-infected milk. The data does not exclude the probability that even in the rare cases in which agglutinins did appear, they were the result of ingesting colostrum or milk containing agglutinins. He stated that it would not be logical to assume that his experimental calves acquired the positive reactions as a result of ingesting infected milk since the blood sera of the calves were not tested until after they had received milk.

In a later report, however, published in 1924, covering the significance of *Bacterium abortum* antibodies in the blood of ten heifer calves and one bull, Huddleson and Hasley[6] quite clearly brought out that the ingestion of colostrum is responsible for the positive complement fixation and agglutination tests obtained in most cases. However, in case 1000 A.3, the development of a positive complement fixation test after nursing is unexplained.

In one other case, the authors state: "The blood serium of N.6A was positive to both tests before nursing thus presenting a clear-cut case of what might be termed prenatal infection." The following data are given on this case:

"N.6 A. Born May 14, 1920, one month premature. Very weak. Fetal membranes retained. Dam previously infected by feeding cultures of *Bact. abortus*. *Bact. abortus* isolated from fetal membranes and uterine exudate at parturition. Milk not examined. Blood of dam positive 1:500 to agglutination test, 0.005 to complement fixation test. Blood of calf positive to both tests before nursing. Nursed dam for three days, then placed on negatively reacting cow for six weeks. Blood remained positive until July 8, 1920."

The positive reaction before nursing was in the first tube only (0.04 mil) and in 0.1 and 0.04 mil to the complement fixation test. When six days old, after nursing the dam three days and then being changed to a negatively reacting cow, its blood was positive with 0.005 mil to both tests.

Prenatal infection of fetuses is well known, but that this infection causes antibodies to appear in their blood has not been established. Reactions in the titre shown by calf N.6 A before nursing are not considered positive in adult animals, and they may have been nonspecific.

Marked discrepancies appeared between the results of the agglutination and complement fixation tests. The reason for this was ascertained during the course of the work and is explained by the use of antigen of too dense a turbidity in the agglutination test. With this we agree, as we have found that the increased turbidity of agglutination test antigen above the ideal standard rapidly decreases the efficiency of the test. The establishment of an ideal standard for the antigen in this test is one of the most important steps to be taken in avoiding discrepancies in its application under various conditions and in different laboratories. We never use an antigen more turbid than that represented by a 3.5 cm. Gates opacimeter reading and one of 5 or 6 centimeters may ultimately prove to be the most acceptable standard.

In 1918, Robinson[12] carried out an experiment with sixteen cows and their calves or fetuses to see whether or not offspring of infected cows showed any production of agglutinins in their blood sera. No positively reacting fetal serum was obtained. One calf, born too weak to stand and suck, showed an agglutination in a dilution of 1 to 25, while its dam was positive in 1 to 500. In two cases the titre of the calf and of the cow serum was the same. In discussing the result of his Experiment No. 3, he states: "In the case of calves born alive and healthy, one may or may not get a weak agglutination, though in a few cases a high one is obtained, but there seems to be no definite ratio between the agglutination of the cow's serum and the calf's."

In the same year, Rettger and White,[11] in a study of infectious abortion in a mixed dairy herd, found that calves became negative to the agglutination and complement fixation tests after the age of two to six months, although shortly after birth they invariably reacted in the same way as the mother. They remained negative until nine to ten months old or the period of sexual development and maturity. After this time whether they were from infected mothers or not, little difference was observed in the development of positive reactions.

Thirty-five daughters of twenty-nine positive cows were listed by the side of forty calves from thirty non-reacting dams over a period of four years (1914 to 1917). Fourteen, or 40 per cent, of the former gave a positive reaction, and seventeen, or 42.5 per cent of the latter, were positive to at least one test. Ten daughters from positive dams aborted, two or them twice, and eleven daughters of negative dams aborted, two of them twice. That the reactions of the dams have no influence on the later history of the calves is clearly brought out in these data.

Seddon[15] reported, in 1919, three cases in calves in which the animals were infected with *Bacterium abortum* without giving any evidence of agglutination reaction. One was a case of a full-term calf born dead from which *Bacterium abortum* was isolated from the stomach contents. A second was a calf expelled alive, at 262 days gestation, from an artificially-infected dam with infected uterus. The third was a calf which, at eighteen days of age, was injected intravenously with 2 mils of living culture emulsion. Thirteen blood tests with blood taken from the third to the seventieth day after the inoculation failed to give a positive reaction. It was then given a second injection at eighty-eight days of age, after which ten blood tests, made from three to fifty-three days after the second injection, all failed to give a

positive reaction. Three other calves inoculated in the same manner, when the second injection was given, developed a positive reaction in eighteen days.

In the same year, Dick[3] reported field observations (begun in 1916) on three herds, in all of which the calves received raw milk from the respective herds until weaned.

In herd A, heifers of breeding age were kept with the main herd, and, in 1918, twenty-two, or 44 per cent, aborted.

In herd B, the young stock, instead of having a community pasture, were kept in a separate lot on the same farm, but the segregation was not absolute. In 1918, two of the twelve head, or 16.6 per cent, aborted.

In herd C, the calves were kept on a separate farm until within two weeks of calving time. Three-year records are given for this farm, as follows:

Year	Abortions	Parturitions
1916	1	18
1917	none	23
1918	none	23

Previous to 1916, a large percentage of the heifers on this farm had aborted each year.

In 1920, Simms and Miller[16] reported experiments in which they had fed forty-six heifer calves milk from infected cows known to contain *Bacterium abortum.* All of these calves were negative to the agglutination test before they reached six months of age. After being bred they were handled in such a manner that they were not exposed to infected cows or premises. At the time of the report, twenty-three had terminated their first pregnancies at full term in a normal manner. Agglutination tests of their blood remained negative throughout the respective gestation periods. The writers suggested that there was a possibility of a quiescent, localized infection in such cases, but believed that were this true, the negative heifers might change to positive after pregnancy had taken place. No evidence of such an occurrence was obtained.

Until this time a thorough understanding of the relationship of colostrum to the presence of antibodies in the blood of new-born calves was lacking. This was clearly brought to the attention of investigators by the work of Little and Orcutt[7] in 1921. For several years previously, these workers had tested the blood sera and transudates of fetuses from time to time to determine the relation between

concentration of abortion agglutinins in the blood sera of mother and fetus. The results showed that even when the serum of the mother had a high agglutination content, little or none was found in the fetal blood.

In experiments covering twenty cows and nineteen calves, the fact was established that even when the blood and colostrum of the dam had a relatively high agglutination content, the blood of the calf, with a single exception, was free. When colostrum was withheld and milk of a lower or negative agglutination titre substituted, agglutinins failed to appear in the calves' blood after several days.

The antibodies in a calf's blood are absorbed from the digestive tract, the rate of absorption being fairly rapid. In slightly over an hour after the feeding of colostrum, they began to appear and had nearly reached their maximum concentration five hours after feeding.

These experiments made it evident that agglutinins for *Bacterium abortum* found in the blood serum of new-born calves are obtained from the mother through the colostrum. Calves at birth, unfed, are without agglutinins.

In 1922, Duebler[2] reported on a group of twenty heifers raised several years previously under identical conditions until two months before breeding. Ten were then placed in barns with the cow herd and the other ten in a barn for young stock only, having no connection with the breeding herd. All were bred. Seventy per cent of the heifers in the cow barn aborted, and 100 per cent of the other group calved normally. From then on to the time of reporting, all heifers on the farm were bred and kept in the heifer barn, and, with an average of fifteen to twenty heifers calving yearly, none had aborted.

Quinlan[10] reported, in 1922, a series of experiments in South Africa in which he used five groups of calves, totalling forty-one head, with one control animal. The infected milk given the calves was from cows whose blood and milk samples gave positive agglutination tests. In some cases, the presence of the organism was actually demonstrated by guinea pig inoculations.

In these experiments the author attempted to determine by the agglutination titre of the blood serum of the calves the effect of the feeding of infected milk. The titre was studied in some cases until the calves reached maturity.

In Group A of eleven head separated from their infected dams and artificially fed infected milk and Group B of four head, born to infected dams and allowed to suckle them, antibodies were found in the blood of five.

In Group C of eighteen head from non-infected dams fed infected milk and in Group D of six head from non-infected dams, but fostered by infected dams, specific antibodies were found in the blood in only three cases.

The author makes the statement that "apparently the infection which results before birth through the infected mother is to be considered in far greater measure the cause of the appearance of the antibodies in the calf's blood than the ingestion of infected milk."

The author cites an interesting case, Friesland Bull 89, one of the two head of Group E. This calf was born from an infected cow and was fed its mother's milk for three days. The milk of this cow had never given a positive reaction to the agglutination test. The calf, when born and when removed from his mother, showed a slight agglutination with .025 mil of serum, but after having been fed non-infected milk for one month and eighteen days, showed an agglutination titre of .001 mil. This, the author states, "appears to be a case of temporary active infection and moreover the infection seems to have been of intrauterine origin."

Despite the finding of agglutination reactions in the early days of the lives of the experimental calves, the author found that the agglutination titre tended to fall and disappear altogether after twelve to fifteen weeks even though infected milk was still being fed regularly.

When antibodies were present at the time feeding infected milk was stopped, they disappeared shortly afterwards. In some cases, calves were fed exclusively with infected milk up to the ninth month, without provoking the appearance of antibodies in their blood. It was observed that the calves remained in excellent condition despite the ingestion of the abortion organisms. The results also prove that calves fed infected milk do not become chronic carriers of the disease. The author was able to follow up the history of nearly all the female calves used in the experiment and in no case found any evidence that an animal become a carrier.

The value of a proper understanding of the agglutination reactions in calves is great, and, we cannot but feel after considering his work that, at the time of preparing his report, the author was not familiar with the very important role played by positive colostrum in this respect as disclosed by the work of Little and Orcutt.[7]

In 1924, Barger and Hayes[1] reported experiments in which *Bacterium abortum* was given in the milk to three groups of two calves each to ascertain whether it could be recovered from the feces. Both naturally and artificially infected milk were used. The organism

was successfully recovered from the feces of the calves and it was also found in the lymph glands of the head for seventeen and nineteen days respectively in Groups B and C after the discontinuance of the infected milk. Agglutinins did not appear in the blood sera of the calves fed with infected milk for periods ranging from fifteen to twenty-one days.

PLAN OF THE EXPERIMENTS

In our study of this problem, thirty-nine calves, including both sexes, were used. They were all born to our experiment cows and were kept under our direct supervision and control from birth until they died, were killed, or terminated their first gestation period. Some of them received colostrum and from others it was withheld.

The infected milk with which these calves were fed contained *Bacterium abortum*, in some instances, when drawn from the udder, but, in all cases, a definite quantity of a known pathogenic strain of the organism was added to the milk before each feeding.

There were some deaths in the calves a few days after they were started on the infected milk. The remainder were slaughtered from seven days to twenty-four months afterwards, the period between the time infected milk was withdrawn and the date of death varying from seven days to eighteen months.

When the calves were slaughtered, cultures were made and guinea pigs inoculated from various body tissues to test for the presence of *Bacterium abortum*.

All calves living longer than six months were weaned at that age. Eleven head were allowed to reach sexual maturity, breed and complete one gestation period, when their colostrum and placentae were examined for the presence of the organism. These animals were bred at about fifteen months of age. A shorter period of time than ordinarily obtains in practice, therefore, elapsed between the last exposure to *Bacterium abortum* infection and the establishment of pregnancy.

Blood samples were collected and agglutination tests made at frequent intervals during the lives of the calves.

PREPARATION OF THE SUSPENSION OF *BACTERIUM ABORTUM* ADDED TO THE MILK

Throughout the work, the strain of *Bacterium abortum* used was our Laboratory No. 80, originally obtained from K. F. Meyer. This was an especially virulent strain of the organism.

From December 5, 1922, to January 25, 1923, each calf that was being fed received daily the growth on one glycerin glucose agar slant culture washed off with about 20 mils of physiological saline solution. One-half of this was added to the morning and the remainder to the evening feed of milk. After January 25, on account of the increased number of calves, the organism was grown on the same medium in Blake bottles. The growth was washed off and the the suspension standardized to 1.5 cm. Gates opacimeter reading. A suspension of this density of the *Bacterium abortum* organism from a 48 to 72-hour culture will show an average of five to six billion organisms per mil by the plate culture method. A half-ounce vial of this suspension was added to the milk for each calf night and morning.

From January 25, 1923, to January 23, 1924, when the last dose of the organism was given to calf 1780, sixty-one batches of the suspension were made up. One guinea pig was inoculated intraperitoneally with 1 mil from each batch except the first, to test its virulency. Fifty-nine of the sixty guinea pigs developed abortion disease. Twenty-six died from the infection and thirty-three were killed approximately six weeks after the date of inoculation. One guinea pig died from intercurrent disease a few days after inoculation.

RESULT OF THE EXAMINATION FOR THE PRESENCE OF *BACTERIUM ABORTUM* IN THE TISSUES OF CALVES RECEIVING THE ORGANISM IN MILK FOR VARYING PERIODS OF TIME

When the calves were slaughtered, two cultures were made, on glycerin glucose agar and cooked blood agar, respectively, from the following tissues with the exceptions that appear in the table: atlantal, submaxillary, posterior pharyngeal, mediastinal, bronchial, gastric, hepatic, mesenteric, superficial inguinal or supramammary, pelvic, prescapular and precrural lymph glands, thymus gland, liver, spleen and stomach and intestinal contents.

From ten to twelve guinea pigs were injected with the tissues of each calf, parts of several glands being inoculated into a single guinea pig in some cases.

Table 1 gives the result of this examination.

In table 1, calf 1732 did not receive infected milk until it was seventeen days old, D–IV calf until it was three days old and calf 1763 until it was forty days old. The last did not receive infected milk during the first six weeks of its life when it was running in pasture with its non-infected dam. Calf 1745 did not receive the organ-

Ear tag No.	Date of birth	Sex	Colostrum	Condition of gastrointestinal tract	Period of ingesting *Bacterium abortum* in milk	Period between withdrawal of *Bacterium abortum* and slaughter	Date o[f] death
1745	Feb. 1, 1923	Female	No	Died of scours	2 days	3 days	Feb. 6,
1757	Mar. 24, 1923	Female	No	Died of scours	2½ days		Mar. 27,
1740	Jan. 20, 1923	Female	No	Died of scours	3½ days	1 day	Jan. 25,
1759	Apr. 1, 1923	Female	Yes	Died of scours	6 days		Apr. 7,
1749	Feb. 7, 1923	Male	Yes	Diarrhea	7 days		Feb. 15,
1732	Nov. 18, 1922	Male	No	Diarrhea	7 days		Dec. 12,
D. IV	Dec. 23, 1922	Female	Yes	Normal	7 days	7 days	Jan. 9,
1746	Feb. 2, 1923	Male	Yes	Very slight diarrhea	7 days	7 days	Feb. 17,
1753	Mar. 2, 1923	Male	Yes	Normal	14 days	7 days	Mar. 24,
1735	Dec. 3, 1922	Male	No	Normal	14 days	7 days	Dec. 26,
1752	Feb. 16, 1923	Male	Yes	Normal	21 days	10 days	Mar. 20,
1733	Dec. 3, 1922	Female	No	Normal	21 days	11 days	Jan. 6,
1750	Feb. 9, 1923	Male	Yes	Normal	4 weeks	3 weeks	Mar. 31,
1734	Dec. 3, 1922	Female	No	Normal	4 weeks	3 weeks	Jan. 23,
1737	Dec. 31, 1922	Male	No	Diarrhea	5 weeks	4 weeks	Mar. 4,
1739	Jan. 20, 1923	Male	No	Normal	7 weeks	7 weeks	Apr. 28,
1741	Jan. 23, 1923	Male	No	Diarrhea	9 weeks	6 weeks	May 8,
1758	Mar. 31, 1923	Male	Yes	Normal	12 weeks	7 weeks	Aug. 13,
1765	Apr. 15, 1923	Male	Yes	Normal	4 months	8 weeks	Oct. 16,
1770	May 7, 1923	Male	Yes	Normal	5 months	9 weeks	Dec. 10,
1768	Apr. 7, 1923	Male	Yes	Normal	4½ months	10 weeks	Dec. 15,
1772	May 19, 1923	Male	Yes	Normal	6 months	11 weeks	Feb. 5,
1780	July 23, 1923	Female	Yes	Normal	6 months	3 months	Apr. 23,
1779	July 22, 1923	Female	Yes	Normal	6 months	6 months	July 22,
1778	*July 18, 1923*	*Female*	*Yes*	*Normal*	*6 months*	*9 months*	*Oct. 20,*
1777	June 29, 1923	Female	Yes	Diarrhea	6 months	12 months	Dec. 26,
1776	June 27, 1923	Female	Yes	Normal	6 months	15 months	Mar. 27,
1775	June 13, 1923	Female	Yes	Normal	6 months	18 months	June 12,
1738	Jan. 17, 1923	Female	Yes	Normal	6 months		
1763	Apr. 16, 1923	Female	Yes	Normal	6 months		
1744	Jan. 29, 1923	Female	No	Diarrhea	6 months		
1755	Mar. 6, 1923	Female	No	Normal	6 months		
1748	Feb. 7, 1923	Female	No	Normal	6 months		
1736	Dec. 20, 1922	Female	No	Normal	6 months	All bred and passed th[…]	
1773	May 23, 1923	Female	Yes	Very slight diarrhea	6 months		
1754	Mar. 4, 1923	Female	No	Diarrhea	6 months		
1769	May 2, 1923	Female	Yes	Normal	6 months		
1767	Apr. 16, 1923	Female	Yes	Normal	6 months		
1751	Feb. 13, 1923	Female	No	Normal	6 months		

NOTE.—+ indicates *Bacterium abortum* present, − indicates *Bacterium abortum* not present, Blank spac[e]

TABLE 1.—DAT

f	Atlantal				1. Submaxillary 2. Posterior pharyngeal					Stomach and conte		
	Guinea pig			Cul-tures	Guinea pig			Cul-tures		Guinea		
	Post mortem	Spleen culture	Blood reaction		Post mortem	Spleen culture	Blood reaction		Post morte	Post mortem	Sple cultu	
1923	+		+		+		+					
1923	+		+	+	+		+	1 −				
1923	+		+	−	1 +		1 +					
1923	+		+	−	+		+	−				
1923	+		+	−	+		+	+	+	Died inoc	8 days ulation	
1922	+	+	+	+	+	+	+	+	−	+	+	
1923	+		+	+	+		+	+	−	Died inoc	2 days ulation	
1923	+		+	+	+		+	+	+	−		
1923	+		+	+	+		+	+	+	−		
1922	+	+	+	+	+	+	+	+	−	−		
1923	−		−	+	+		+	1 + 2 −	+	−		
1923	−	−	−	−	+		+	+	−	+		
1923	−	−		−	−		−	1 − 2 +	−	−		
1923	+		+	−	−	−	−	+	+	Died inoc	2 days ulation	
1923	−	−	−	+	−		−	1 − 2 +	−	−		
1923	−		−	−	−		−	−	−	−		
1923	−		−	−	−		−	−	−	−		
1923	+	+	+	−	−	−	−	−	−	−	−	
1923	−		−		−	−	−		−	Died inoc	day aft ulation	er
1923	−		−	−	−		−	−	−	−		
1923	−		−	−	−		−	−	−	−		
1924	−	−	−	−	−	−	−	1 + 2 −	−	−	−	
1924	−	−	−	−	−	−	−	−	−	Died inoc	2 days ulation	aft
1924	−	−	−	−	−	−	−	−	−	−	−	
1924	−	−	−	−	−	−	−	−	−			
1924	−	−	−	−	−	−	−	−	−			
1925	−	−	−	−	−	−	−	−	−			
1925	−	−	−	−	−	−	−	−	−			

rough one gestation period.

ism during the last three days of its life because it was given little milk on account of severe diarrhea. When cultures are marked negative, it signifies that *Bacterium abortum* was not recovered. The cultures were not always sterile. In some cases, where the calves died of scours *B. coli* overgrew the tubes.

In addition to the tissues listed in the table, cultures were also made in fifteen cases from the testicle, in nine from the uterus, in eight from the kidney, in eight from the epididymis, in six from the vagina, in four from the heart, in three from the gall bladder and in two from the lung. All resulted negatively, except one culture from the epididymis of calf 1753, which developed *Bacterium abortum.*

Two of the animals were pregnant when killed. No. 1778 was about six weeks and No. 1775, five months along in gestation. The fetus and membranes from No. 1778 were triturated in a mortar with saline solution and injected into one guinea pig. From the much further developed fetus of No. 1775, three guinea pigs were injected from the maternal and fetal cotyledons; lung, liver and spleen extract, and stomach and intestinal contents, respectively. All resulted negatively.

Two cultures were also made from the lung, liver, spleen, and stomach contents, respectively of the fetus from No. 1775 and remained sterile except for a mold on one tube and a few large cocci on another.

Twenty eight calves died or were killed and their tissues examined in the above manner, the remaining eleven being allowed to complete one gestation period.

The table shows that *Bacterium abortum* ingested in milk passes through the walls of the digestive tract and gains access to various lymph glands and other organs, particularly the spleen. In no case was the organism found in the thymus gland.

The lymph glands about the head became infected with great regularity, showing that *Bacterium abortum* is taken up from the mouth and throat. It is also present, as would be expected, in the lymph glands along the digestive tract. However, its presence in the bronchial and mediastinal glands was quite commonly observed. In the more remote body glands, it can rarely be demonstrated. The findings show that the organism can undoubtedly pass through the intact mucous membrane of the digestive tract, be taken up by the lymphatic glands and, in some cases, must gain access to the blood stream from which it may occasionally be deposited in almost any tissue of the body.

Whether the calf receives colostrum or not seems to have no bearing on the invasion of the organism. Despite its constant penetration and possibilities for quite general distribution, there does not seem to exist in either the male or female calf any location that furnishes a favorable place for more than temporary existence in the absence of a constant supply of fresh infection.

A few weeks after infected milk is withdrawn, the organism is rarely demonstrated and undoubtedly in the great majority of cases under natural conditions the bodies of calves are free from the organism in from six to eight weeks after the cessation of infection.

Among our experiment animals where very heavy dosing had occurred, No. 1758 was found to contain the organism in the atlantal gland at the time of slaughter, seven weeks after infection had ceased, and it was recovered culturally from the submaxillary lymph gland of calf 1772 when killed, eleven weeks after the last infection. Six of the animals, including the two mentioned above that became pregnant, were examined after living longer following the removal from sources of infection, and all were negative.

AGGLUTINATION REACTIONS OF THE BLOOD OF CALVES TO *BACTERIUM ABORTUM* ANTIGEN

The effect of the ingestion of the milk or colostrum and milk containing *Bacterium abortum* on the agglutination reactions of the calves has been carefully observed by taking blood samples at regular intervals throughout the two and one-half years covered by the experiment. This is given in table 2.

It will be observed by reference to this table that, despite the excessive quantity of virulent *Bacterium abortum* being ingested daily by the calves, their agglutination tests were generally negative.

Colostrum was withheld from some of the calves with two separate objects in view. The first was to study any difference in the invasive ability of the organism and the agglutination titre of the blood as compared with that of the dams. The second was to ascertain the effect of the presence or absence of colostrum ingestion on the development of gastro-intestinal disturbances.

When colostrum was withheld from calves, an attendant was present at time of birth. In case any doubt existed as to the calf possibly having received colostrum, it was allowed to remain with the dam and was placed in the group that received colostrum.

During the first ten days of their lives, calves in the no-colostrum group were fed milk from cows with negative agglutination titre and

that had been fresh for a period of at least seventeen days. Only No. 1736 received milk from cows this early in lactation. It was born on December 20, 1922, and received milk that night from cows 2312 and 26 which had both freshened on December 3, 1922, and this was the only milk available. It was recognized that in case gastro-intestinal disturbances were going to occur, they would develop in the great majority of cases during this period. After the ten days had elapsed, their feeding could not be watched so closely from this standpoint on account of the number of calves being handled and the availability of the desired kind of milk. Also, our chief concern at this time consisted in seeing that *Bacterium abortum* was only ingested by the calves that we desired to have it and not by those that were being kept for periods of time after its withdrawal before slaughter and examination.

Some of the calves received milk indefinitely from cows fresh thirty days or longer, but, in these cases, the possibility that they received milk from the same bucket that had previously contained positive colostrum or milk without rinsing was quite likely to have occurred.

Sixteen calves received no colostrum. The first blood sample of all of these calves was negative, except that of No. 1751, which was positive in only the .02 mil tube and negative in .04 mil, .01 mil and .005 mil on the day after its birth. This reaction is paradoxical: the blood of the dam was negative—our laboratory records show it was not rechecked, and faulty technique may have been responsible. Of the sixteen dams of these calves, nine showed a positive agglutination reaction in a titre of .04 mil to .01 mil, the remaining seven were negative.

In the case of the twenty-three calves which received colostrum, nearly all were from negative dams. Only Nos. 1759 and 1749 deserve mention. The blood of these calves showed absence of agglutinins when tested five and seven days respectively after birth from cows that showed a positive blood agglutination in a titre of .02 mil and .01 mil respectively. One of these, No. 1759, was dead of scours at the time the blood was taken. Unfortunately we did not test the agglutination titre of the colostrum of these cows and it may be that it was very low or negative in both cases. That ingestion of positive colostrum by calves will quickly result in agglutinins being present in their blood is very easily demonstrated.

In studying table 2, it will be observed that in some cases the titre of the blood of the calves changed from negative at one test to positive with .005 mil of serum at the next. This may have been

due to the fact that colostrum or milk having a high agglutination titre was fed to these calves in the interim between the tests. However, the probability of an occasional temporary active production of agglutinins within the calf as a result of the ingestion of large quantities of *Bacterium abortum* must be considered.

In this connection we would mention particularly Calf 1736. This animal was born December 20, 1922, to Dam 2404, showing a suspicious blood agglutination test but in which *Bacterium abortum* could not be demonstrated at time of parturition. It was immediately removed from its dam without getting colostrum and fed with milk from two negative cows, plus the *Bacterium abortum* suspension, until March 23, when it was given milk from a positive cow. On December 27, 1922, when seven days old, its blood was entirely negative. On January 8, 1923, when nineteen days old, its blood was very positive with .01 mil and partially so with .005 mil. On January 17, 1923, when twenty-eight days old, it had again become entirely negative. This case may be taken as evidence that *Bacterium abortum* ingested with milk stimulated the very temporary production of antibodies in the blood. Even if it is the proper explanation of the rise in agglutination titre, of the other cases in table 2 it is of irregular occurrence and its duration covers a short period of time.

The possibility of intra-uterine infection having a bearing on the presence of agglutinins in the early life of calves has attracted our attention principally as a result of the following statement by Little and Orcutt[7] in their work in demonstrating positive colostrum to be the carrier of agglutinins to new-born calves: "The problem of the production of agglutinins by the fetus in whose tissues *B. abortus* has multiplied and which is subsequently expelled prematurely is not touched by these observations."

We have run agglutination tests on the blood of twenty-one fetuses in the bodies of which we have demonstrated *Bacterium abortum* by cultural methods and guinea pig inoculations. In every instance these tests were entirely negative in the four dilutions of .04 mil, .02 mil, .01 mil and .005 mil, respectively. The result of this work is tabulated in table 3.

We are of the opinion that this series of tests corroborates the work of others and demonstrates that the pentration of the tissues of a fetus by *Bacterium abortum* does not result in the production of agglutinins by the fetus in utero.

TABLE 3.—SHOWING NEGATIVE BLOOD REACTIONS OF FETUSES INFECTED WITH *BACTERIUM ABORTUM* IN UTERO

No. of fetus	Period of gestation	Agglutination test of blood	Tissues from which *Bacterium abortum* was isolated: Lung		Liver		Spleen		Stomach and intestinal contents	
			Culture	G. P.	Culture	G. P.	Culture	G. P.	Culture	G. P.
7	9 months........	− − − −	−	+	−		−		−	+
10	7 months........	− − − −	+	+					−	+
11	7 months........	− − − −	+	+	−				+	+
12	7 months........	− − − −	+	−					+	+
13	8 months........	− − − −	+	+					+	+
14	7 months........	− − − −	+	+					−	+
15	8 months........	− − − −	+	+					+	+
18	7 months........	− − − −	+	+					+	+
20	7 months........	− − − −	+	+					+	+
25	8 months........	− − − −	+	+					+	+
35	8 months........	− − − −	+	+					−	−
37	9 months........	− − − −	+	+					+	+
41	6 months........	− − − −	−	+	−	+	+	+	+	+
46	8 months........	− − − −	+	+	−	+	+	+	+	+
49	8 months........	− − − −	+		−		−		+	+
50	9 months........	− − − −	+	+	−	+	−	+	+	+
53	8 months........	− − − −	−	+	−	+	−	+	−	−
57	7 months........	− − − −		+		+		+		+
A.E.IV	5½ months......	− − − −	−	+	+	+	+	+	+	+
A.E. V	6½ months......	− − − −	+	+	+	+	−	+	+	+
A.E.VI	6 months........	− − − −	−	+	−	+	+	+	+	+

EFFECT OF THE SUBCUTANEOUS INJECTION OF LIVE AND DEAD SUSPENSIONS OF *BACTERIUM ABORTUM* ON THE AGGLUTINATION TITRE OF THE BLOOD OF CALVES

On account of the failure of *Bacterium abortum* infection of fetuses in utero and also the failure of the ingestion of excessive quantities of the organism in milk from birth to six months of age to produce agglutinins in the blood of calves, we carried on a few experiments to see with what regularity subcutaneous injection of live and dead organisms would cause the development of these bodies in the blood of the injected animal.

The first test involved only one calf, born October 4, 1924, to a negative dam. This calf was injected subcutaneously on the side of the neck with 10 mils of a suspension of *Bacterium abortum*. having a .9 cm. Gates opacimeter reading on October 30 and again on November 20, 1924. Blood was drawn from the calf on October 29, November 5, 10, 17 and 25 and December 2, in all of which it was

completely negative. On November 25, a local swelling 4 inches in diameter was present where the injection of the organism had been made November 20, 1924.

On December 22, 1924, blood was taken from the calf again and showed a positive reaction in the first tube titre .04 mil. In looking for an explanation of this reaction, it was found that the attendant had milked into his pail a positive cow, which calved December 1, 1922, had emptied the milk out and then, without rinsing the pail, had proceeded to milk the negative cow supplying the calf. The blood of the positive cow had a high agglutination reaction and her colostrum was positive at a titre of .0015 mil. This contaminated pail had been used several times between December 2, when the last negative blood sample was taken from the calf, and December 22, when the blood of the calf showed a positive reaction in the first tube. While the low titre agglutination might have been the beginning of a reaction from the injected organisms the experiment was terminated.

This calf had received two injections of live organisms and a period of thirty-three days had elapsed from the first one without showing any reaction, although it is quite well established in adult cows that, with subcutaneous injection, seven to ten days are sufficient to produce a well marked, positive reaction in a titre of .01 mil or higher.

On March 30, 1924, four more negative calves were selected to receive a killed suspension of the *Bacterium abortum* organism, of the same dose and density as that used with the first calf. These calves were all injected March 30, April 14 and April 28. The following blood reactions were obtained:

No.	Date of birth	Feb. 27, 1925	Mar. 30, 1925	Apr. 15, 1925	Apr. 28, 1925	May 11, 1925
1831	Jan. 12, 1925	– – – –	– – – –	‡‡‡∓	‡‡‡‡	‡‡‡‡
1833	Jan. 5, 1925	– – – –	– – – –	‡‡±∓	‡‡‡‡	‡‡‡‡
1835	Jan. 18, 1925	– – – –	– – – –	– – – –	‡‡‡‡	‡‡‡‡
1838	Feb. 5, 1925	– – – –	– – – –	‡‡‡–	‡‡‡‡	‡‡‡‡

With the development of the very positive reaction in all four of the animals, this experiment was terminated.

On May 11, 1925, another series of four calves was selected to receive live organisms of the same dose and density as used in the first experiment. Three of them were negative and one was positive as a result of nursing a positive dam. This calf was kept in the group

purposely. Each of these calves was injected subcutaneously with the organisms on May 11, May 27 and June 14. The following blood reactions were obtained:

No.	Date of birth	Apr. 27, 1925	May 11, 1925	May 27, 1925	June 14, 1925	June 30, 1925
1842	Apr. 21, 1925		----	----	‡++∓	‡‡+∓
1843	Apr. 11, 1925	----	----	----	‡‡‡‡	∓‡‡±
1844	Apr. 9, 1925	--±‡	∓‡‡+	+±±-	‡+++	+++-
1845	Feb. 27, 1925	----	----	----	‡‡‡+	‡‡‡‡

It would, therefore, seem that either live or dead *Bacterium abortum* organisms injected subcutaneously will result in the development of specific agglutinins in the blood of the calf. They are somewhat slower in appearance than with adult animals.

EFFECT ON THE MORTALITY AND MORBIDITY OF CALVES OF FEEDING AS COMPARED TO THE WITHHOLDING OF COLOSTRUM

The work of Smith and Little[17] on the importance of colostrum to the new-born calf clearly demonstrates the value of this secretion in preserving its health. We were desirous of ascertaining if all calves in our experiment receiving colostrum would remain healthy and also if it were at all practical to raise calves from which it was completely withheld during the early period of their lives.

In addition to the thirty-nine calves listed in table 1, ten others were placed in the experiment, but died at such times that they were not recorded in the *Bacterium abortum* studies. There was thus a total of forty-nine head of calves used. Twenty-seven of these received colostrum and twenty-two did not. We observed equal precautions in the feeding of both groups to prevent digestive disturbance and changed the feeding procedure in various ways in an attempt to remedy any abnormal conditions that developed.

The calves were started on small quantities of milk, diluted with water in some cases, and the amount fed gradually increased. During the first few days, the feed was usually given three time a day. In cases where gastro-intestinal disturbances developed, milk was entirely withdrawn for as long as twenty-four hours and barley water substituted. The actual feeding records of calf 1777 in the colostrum group and 1737 in the no-colostrum group are given below as typical.

Calf 1777, born June 29, 1923. Received colostrum from dam June 29 and 30, and morning of July 1.

Date	Morning and Evening (each)	Noon
July 1	1 quart milk (evening only)	
July 2	1 quart milk	1 quart milk
July 3	1½ quarts milk	1 quart milk
July 4 to 7, inc.	2 quarts milk	1 quart milk
July 8	Showed scours	
	1 quart barley water	1 quart barley water
July 9	1½ quarts milk	1 quart milk
July 10 and 11	2 quarts milk	1 quart milk
July 12 and 13	2½ quarts milk	
July 14 to 19, inc.	3 quarts milk	
July 20 to 25, inc.	3½ quarts milk	
July 26 to Aug. 19, inc.	4 quarts milk	
Aug. 20	Showed scours	
	2 quarts milk (evening only)	
August 21	2 quarts milk (morning)	
	4 quarts milk (evening)	
Aug. 22 to Nov. 30, inc.	4 quarts milk	
December 1 to 28, inc.	Gradually decreasing amount of milk until weaned	
December 29	Weaned	

Calf 1737, born December 31, 1922. Immediately removed from dam without getting colostrum.

Date	Morning and Evening (each)	Noon
December 31	¾ quart milk and	
	¼ quart water	
	(evening only)	
January 1	½ quart milk	
	¼ quart water	
January 2	Started to scour	
	½ quart milk, ¼ pint water (morning)	½ quart milk
		½ quart water
	½ quart barley water (evening)	
January 3	½ quart milk	½ quart milk
	½ quart water	½ quart water
January 4	¾ quart milk	¾ quart milk
	½ quart water	½ quart water
January 5 to 12	1 quart milk	1 quart milk
	½ quart water	½ quart water
	Evening of January 11 calf would not eat, but apparently not sick.	
Jan. 13 to Feb. 10, inc.	1 quart milk	1 quart milk
	½ quart water	½ quart water
February 11 to 19, inc.	1½ quarts milk	1½ quarts milk
February 20 to 22, inc.	3 quarts milk	
Feb. 23 to March 4	4 quarts milk	
March 4	Killed.	

Three of the calves receiving colostrum, Nos. 1759, 1761 and 1764, died during the first few days of life from scours. Four others, Nos. 1749, 1746, 1777 and 1773, showed some evidence of scours but it did not terminate fatally. Twenty remained normal.

Eight of the calves which did not receive colostrum died of scours, Nos. 1745, 1747, 1757, 1740, 1743, 1756, 1760 and 1768. Six others, Nos. 1732, 1737, 1741, 1744, 1754 and 1742, showed evidence of scours but recovered. No. 1742 died later from poisoning. The remaining eight developed no gastro-intestinal disturbances.

This tabulation gives the feeding of colostrum a decided advantage in preserving the health of the calf in early life. However, it also shows that this is not the only essential factor in all cases in preventing intestinal disturbances as evidenced by the deaths in calves which received it. This same observation is made in the practical raising of calves in dairy ranches in this state. We have seen one serious outbreak of scours in calves where it was the practice to leave the calf with its dam for a period of several days to one week or longer.

RESULT OF EXAMINATION OF PLACENTAE AND COLOSTRUM FOR *BACTERIUM ABORTUM* IN CASES WHICH CALVED

Ten of the eleven heifers that were allowed to reach maturity and breed carried their calves to term. The result of the examinations of their placentae and colostrum is given in table 4.

These heifers became pregnant at from thirteen to nineteen months of age. All were bred by bulls 411 and 412, which had been used in our original series of abortion experiments, and, therefore, during 1922 and 1923 had been associated with some aborting cows and cows that had been vaccinated with living *Bacterium abortum*. Neither bull became infected by this association and their blood gave negative reactions to the agglutination test. Five services were required to get No. 1736 pregnant. In the first four of these, bull 412 was used and, at the time, he was neither a very active nor sure breeder. The first breeding with bull 411 was successful in getting the animal pregnant. Three services were required to establish pregnancy in No. 1751, the first two being with bull 412, and two for No. 1767, both with bull 411, the remainder conceiving with the first service.

Eight of the ten heifers calved normally. No. 1738 started to calve during the day of November 16, 1924, but was not seen by the attendant until 5 p. m., when she was found lying down with the posterior end of the calf protruding from the vagina. As the attendant rode up to her on a saddle horse, she got up and parturition was

TABLE 4.—BREEDING AND CALVING DATES OF THE ANIMALS ALLOWED TO REACH MATURITY AND RESULTS OF GUINEA PIG INOCULATIONS WITH PLACENTAE AND COLOSTRUM FOR THE PRESENCE OF *BACTERIUM ABORTUM*

Ear tag No.	Date of birth	Breeding date	Approximate age when conception occurred	Calving date	Guinea pigs injected placenta	Guinea pigs injected colostrum	Guinea pigs killed	Blood reaction of guinea pigs	Post mortem of guinea pigs	Cultures from spleens of guinea pigs	Placenta
1738	Jan. 17, 1923	Feb. 10, 1924	13 months	Nov. 16, 1924	4153-54	4155-56	Jan. 6, 1925	—	—	—	Manually removed. Not adherent.
1744	Jan. 29, 1923	May 13, 1924	15½ months	Feb. 18, 1925	4359-60	4361-62	Apr. 3, 1925	—	—	—	Expelled normally.
1755	Mar. 6, 1923	June —, 1924	14 months	Mar. 9, 1925	4392-93	4394-95	Apr. 20, 1925	—	—	—	Expelled normally.
1748	Feb. 7, 1923	June 17, 1924	15½ months	Mar. 21, 1925	4433-34	4431-32	4433-34 died. Others killed May 4, 1925	4433-34 not posted. Others —	4433-34 not posted. Others —	4433-34 not posted. Others —	Expelled normally.
1736	Dec. 20, 1922	Apr. 30, May 21, June 13, July 4 and July 25, 1924.	19 months	May 1, 1925	4534-35	4536-37	June 11, 1925	—	—	—	Expelled normally.
1773	May 23, 1923	July 31, 1924	14 months	May 3, 1925	4538-39	4540-41	June 15, 1925	—	—	—	Expelled normally.
1754	Mar. 4, 1923	July 31, 1924	17 months	May 6, 1925	4552-53	4550-51	4553 died. Others killed June 18, 1925	4553 not posted. Others —	4553 not posted. Others —	4553 not posted. Others —	Expelled normally.
1760	May 2, 1923	Aug. 19, 1924	15½ months	May 23, 1925	4574-75	4572-73	July 8, 1925	—	—	—	Expelled normally.
1767	Apr. 16, 1923	July 25 and Aug. 27, 1924.	16½ months	June 1, 1925	4588-89	4586-87	July 14, 1925	—	—	—	Expelled normally.
1751	Feb. 13, 1923	May 29, July 21, Sept. —, 1924.	19 months	July 1, 1925	4665-66	4663-64	Aug. 13, 1925	—	—	—	Expelled normally.

completed, but the calf was dead. The heifer was quite thin and we think parturition had been slow, the posterior presentation resulting in the calf being smothered during birth.

No. 1748 developed a marked case of dystocia, and embryotomy had to be performed to extract the fetus. She came in labor about 6:30 p.m. March 20, 1925. At midnight, the attendant decided the calf could not be expelled on account of its abnormal presentation. Early the following morning, an examination disclosed the head bent down between the front legs and held by the brim of the pelvis. On account of the small size of the animal, it was necessary to remove both front legs before the head could be elevated and the calf extracted. The placenta came away with the calf. The cow developed metritis and died March 24, 1925.

Laboratory examinations of the dead calves born to heifers 1738 and 1748, respectively:

Calf of heifer 1738:
- Brought to laboratory November 17, 1924.
- Fully developed.
- Externally normal.
- Post-mortem examination, normal.
- Cultures made on cooked blood agar, one tube, and glycerin agar, one tube, from each of the following organs:
  - Lung. No growth visible.
  - Liver. No growth visible.
  - Spleen. No growth visible.
  - Stomach contents. No growth visible.
  - Intestinal contents. No growth visible.
- Guinea pig 4151 was inoculated with lung, liver and spleen extract, and guinea pig 4152 with stomach and intestinal contents. Both were killed January 6, 1925, and were normal. Their blood serum was negative to the agglutination test and cultures made from their spleens remained sterile.

Calf of heifer 1748:
- Brought to laboratory March 21, 1925.
- Externally normal except for fact its two front legs had been removed.
- Post-morten examination, normal.
- Cultures were made on cooked blood agar, one tube, and glycerin agar, one tube, from each of the following tissues:
  - Lung. Bact. coli.
  - Liver. Bact. coli.
  - Spleen. No visible growth. Suspicious Gram negative bodies in smear from this tube. Tissue suspension injected into guinea pig 4525 April 17, 1925. Killed June 3, 1925. Normal. Blood, negative. Spleen culture, sterile.
  - Stomach contents. Bact. coli.
  - Intestinal contents. Bact. coli.

Guinea pig 4435 was inoculated with stomach and intestinal contents and guinea pig 4436 with lung, liver and spleen extract. Guinea pig 4436 died the following day but no post-mortem was made. Guinea pig 4435 was killed May 4, 1925, and was normal. Its blood serum was negative and a cooked blood agar culture from its spleen was negative.

The guinea pigs inoculated with the placenta and colostrum from all of the ten animals were negative for *Bacterium abortum* (see table 4).

ABORTION OF NON-SPECIFIC CAUSE IN ONE ANIMAL

The eleventh animal, No. 1762, of the group that was to complete one gestation period, aborted in the seventh month of pregnancy. This heifer was bred July 1, 1924, and conceived. On January 22, 1925, she was observed in the pasture gaunt and showing evidence of udder development. Suspecting abortion, the attendant searched the pasture and found the fetus and placenta. With this animal at the time were all the other heifers of this group, except No. 1738, which had calved in November and been removed. This heifer was then taken from the pasture. Colostrum was collected from her udder and, together with the fetus and membranes, was brought to the laboratory.

The fetus was normal, but the placenta was very dry and appeared to be shrivelled. Two cultures were made on glucose glycerin agar from each of the following tissues of the fetus:

Heart, liver, lung, spleen,
stomach contents, meconium rectum.

All of these cultures remained sterile and were discarded after three weeks' incubation.

The following guinea pigs were injected intraperitoneally with material from the case:

No. 4291—Lung, liver and spleen of fetus.

No. 4292—Stomach contents and meconium of fetus.

No. 4293—Placenta.

No. 4294—Colostrum.

No. 4293 was dead the second day after inoculation with generalized peritonitis. Cultures made from the viscera developed Bact. coli.

The remaining three guinea pigs were killed March 4, 1925, and were normal throughout. Their blood was negative to the agglutination test and cultures made from their spleens remained sterile.

The blood of this heifer had been negative to the agglutination test as shown in table 2. This case has previously been reported by Traum and Hart[18] as a case of abortion without demonstrable cause.

CONCLUSIONS

1. This series of experiments demonstrates that *Bacterium abortum* ingested by calves with milk find their way to the lymph glands along the alimentary canal, particularly those about the head, and, in some cases, gain access to the blood stream as evidenced by their frequent recovery from the spleen.

2. These organisms do not remain permanently located in the body tissues of the calf. A few weeks after infection ceases, the organisms are no longer found.

3. The longest period in which they were found after infection ceased was seven weeks in one case in the atlantal lymph gland and eleven weeks in another case in the submaxillary lymph gland.

4. No difference between male and female calves was observed in this connection. The feeding or withholding of colostrum also had no effect on the result.

5. Although the period between the time infected milk was withdrawn and pregnancy was established in the animals that were allowed to reach maturity (seven to thirteen months) was much shorter than normally occurs in dairy farm practice, none of them showed any evidence of *Bacterium abortum* infection.

6. The ingestion by calves of large quantities of virulent *Bacterium abortum* organisms in milk, followed by their gaining access to certain lymph glands and other body tissues, including the blood stream, does not with occasional exceptions result in the production of agglutinins in the blood of these animals.

7. The testing of the blood of calves up to the time they are from nine to twelve months of age is, therefore, of no value in herds where the disease is being controlled by the agglutination test and isolation of reactors.

8. Greater morbidity and mortality were experienced among the calves that did not receive colostrum.

9. Nevertheless, we were able to raise calves without colostrum and its feeding did not, in all cases, stop the development of serious and even fatal gastro-intestinal disturbances.

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